Microgravity and the implications for wound healing

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ABSTRACT

Wound healing is a sophisticated response ubiquitous to various traumatic stimuli leading to an anatomical/functional disruption. The aim of present article was to review the current evidence regarding the effects of microgravity on wound healing dynamics. Modulation of haemostatic phase because of alteration of platelet quantity and function seems probable. Furthermore, production of growth factors that are released from activated platelets and infiltration/function of inflammatory cells seem to be impaired by microgravity. Proliferation of damaged structures is dependent on orchestrated function of various growth factors, for example transforming growth factors, platelet-derived growth factor and epidermal growth factor, all of which are affected by microgravitational status. Moreover, gravity-induced alterations of gap junction, neural inputs, and cell populations have been reported. It may be concluded that different cellular and extracellular element involved in the healing response are modified through effect of microgravity which may lead to impairment in healing dynamics.

Key words: Cytokine • Growth factor • Microgravity • Wound healing

Key Points

- regardless of the specific aetiology and external manifestation, the similar array of events that follows the injury is directed at restoration of the original status of wounded tissue
- considering the probability that traumatic injuries will occur during space travel and the importance of efficient repair to the astronaut, it will be crucial to understand the impact of microgravity on various elements and phases of wound healing
- in the present article, we review the current information about the modulation of healing by gravity

INTRODUCTION

Wounds involve the gross macroscopic or subliminal microscopic damage to the anatomical and functional integrity of live tissues.(1,2) The diverse clinical manifestations of injury range from conspicuous cutaneous injuries (3) to scenarios involving subtle metabolic microangiopathies (4). Regardless of the specific aetiology and external manifestation, the similar array of events that follows the injury is directed at restoration of the original status of wounded tissue. The repair process progresses through several overlapping phases, which include inflammation, proliferation and remodelling (2,3). Alteration of any of the interrelated elements involved in healing can affect the entire process and the final outcome (5–8).

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The modern era has witnessed a substantial rise in quantity and quality of manned space flights and has provided promise for the eventual long-term inhabitation of space, either on stations or other planets. Within space, the dynamic equilibrium of human body is altered by exposure to a variety of altered circumstances (9,10). These include changes in gravitational status, neuroimmunoendocrine modulations, modified environmental stimuli (such as radiation), etc. (10). Considering the probability that traumatic injuries will occur during space travel and the importance of efficient repair to the astronaut, it will be crucial to understand the impact of microgravity on various elements and phases of wound healing. In the present article, we review the current information about the modulation of healing by gravity.

Haemostasis

Immediately after wounding, two interrelated pathways are activated in order to stop bleeding. A fibrin clot forms, filling the anatomical void, while platelets contact the exposed

collagen and undergo aggregation and activation. The resulting fibrin/platelet plug contributes to the initial stability of wound and also serves as a provisional matrix that will be replaced as healing proceeds (11,12). Beyond their haemostatic function, platelets release several growth factors such as transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factors and epidermal growth factor (EGF), all of which may play a role in the initiation of healing response (13,14).

Studies that examine the effects of microgravity on haemostatic pathways are contradictory, as reports of both increased thrombotic disease (15) and increased haemorrhagic status (16) can be found in the extant literature. Thrombocytopenia, an increase in activated partial thromboplastin time and defective cell–cell adhesion have been described to occur in the microgravitational condition, increasing the haemorrhagic risk. The sustained elevation of blood pressure that has been shown to result because of microgravity exposure may also increase the threat of haemorrhage (17–21). In contrast, in circumstances of altered gravity, thrombotic incidents may increase because of alterations in blood volume, blood viscosity, elevation of superoxide anions, increases in catecholamine release and metabolic disorders (22–25). Coagulative imbalances have also been reported (26). Fibrin structure, including the thickness of the fibres, the number of branch points, porosity and permeability, have also been suggested to be important determinants of healing outcomes (12). It has been reported that microgravity-formed fibrin gels are more uniform than those formed at normal gravity, although the fibre diameter and matrix porosity are not affected (27) In contrast, later studies showed diminished matrix porosity in microgravitational status (28). The relative inconsistency of data may partially reflect the difference in experimental methods and environments. One possibility is that the diminished platelet content of the platelet/fibrin plug owing to microgravity may reduce contractile force and promote the fibrinolysis rate of fibrin clot (29–31). An earlier loss of superficial eschar because of hastened fibrinolysis would be expected to lead to increased wound contraction (32). Independent of whether haemostasis is altered, bleeding from the wound site under situations of microgravity would permit the formation of large fluid dome because of high surface tension of blood (33). While control of this bleeding pattern may not be difficult (33), special training for providing medical care to wounds may be needed because of altered fine motor skill (34).

Inflammation

Degranulation of activated platelets is an early event in wound healing. Platelets release a multitude of potential mediators at the wound site, including TGF-β, PDGF and EGF, and many of these mediators may modulate the subsequent healing process. PDGF can initiate the chemotaxis of neutrophils, smooth muscle cells and fibroblasts. This growth factor is mitogenic for fibroblasts and arterial smooth muscle cells, can facilitate the release of other growth factors and is also reported to enhance the effects of some growth factors like TGF-β (35–37). Similarly, TGF-β isoforms (TGF-β1, TGF-β2 and TGF-β3) can attract neutrophils to sites of injury (38). The most important role of TGF-β is probably the regulation of the deposition of extracellular matrix components, which occurs through its influence on the proliferation of fibroblasts and their synthetic activity (39). Moreover, TGF-β and PDGF enhance the effect of each other through synergistic interactions (36,37,40). A similar synergistic interaction has been reported for EGF and PDGF (41). While platelets certainly provide an initial pool of EGF, TGF-β and PDGF, other cell types within the wound continue to produce these mediators as healing progresses (42–50).

Space flight probably has the potential to greatly alter the production of EGF, PDGF and TGF-β in wounds. The microgravitational status has been shown to influence the expression the EGF receptor, and EGF-induced signal transduction is impaired in microgravity (51–53). The production of TGF-β has been found to be downregulated in simulated microgravitational status, and microgravity induces the downregulation of the PDGF receptor by 62% (54). Furthermore, the response of wounds to PDGF during space flight was attenuated compared with control wounds on the ground (55). Together, these data suggest that the production of growth factors that are released from activated platelets seems to be impaired by microgravity.

The inflammatory phase involves the regional activation of immune system and the infiltration of wound site by inflammatory cells. Many of the infiltrating immune cells secrete growth factors that stimulate the proliferation of cellular components of the tissues. Neutrophils

Key Points

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- the relative inconsistency of data may partially reflect the difference in experimental methods and environments
- independent of whether haemostasis is altered, bleeding from the wound site under situations of microgravity would permit the formation of large fluid dome because of high surface tension of blood
- while control of this bleeding pattern may not be difficult, special training for providing medical care to wounds may be needed because of altered fine motor skill
- space flight probably has the potential to greatly alter the production of EGF, PDGF and TGF-b in wounds
- these data suggest that the production of growth factors that are released from activated platelets seems to be impaired by microgravity

Key Points

- beyond cell number, functional attributes of leucocytes that are critical to cell migration also appear to be altered by space flight
- several studies suggest that mast cells influence inflammation and repair at sites of injury and a link between increased mast cell content and the formation of hypertrophic scars has been suggested
- comprehensive research aiming at the elucidation of the function and number of these cells in microgravity-exposed wounds seems necessary

Table 1 The effect of microgravity on various elements involved in wound healing

Target	Increase	Decrease	Unaffected
Platelets		+ (16)	
Superoxide anions	+ (22–25)		
Fibrin porosity		+ (28)	+ (27)
Fibrinolysis	+ (29–31)		
EGF function		+ (51–53)	
TGF-β		+ (54,85,86)	+ (87)
PDGF function		+ (54,55)	
Monocytes		+ (59)	
Neutrophils		+ (60,61)	+ (62)
T cell		+ (72–75)	
IL-2		+ (72,74,80)	
IL-1β		+ (79)	
IL-6	+ (80–81)		
TGF-α		+ (100–103)	
Gap junctions		+ (113)	+ (114)
Stem cell activity	+ (129)	+ (127–129)	

EGF, epidermal growth factor; TGF, transforming growth factor; IL, interleukin.

are the first of the circulating inflammatory cells to arrive at the site of injury (56,57). Thereafter, monocytes gradually arrive, eventually becoming the dominant inflammatory cell population in wounded region. It has been suggested that the abundance of monocytes, precursors of macrophages, appears to be a rate-limiting parameter in tissue repair (58). In this context, space-induced deficiencies in monocytes may be critical. Indeed, Taylor *et al*. (59) reported a decrease in peripheral blood monocytes after space flight. Likewise, Ichiki *et al*. (60) reported microgravity-induced neutrophilia. This finding may partially reflect the effect of flight-induced psychological stress on immune function (61). In contrast, Meehan *et al*. (62) reported a post-flight increase in circulating monocytes and no significant changes in plasma cortisol levels. Allebban *et al*. (63) reported a significant reduction in the absolute number of lymphocytes and monocytes and a slight increase in the absolute number of eosinophils and neutrophils after space flight. Thus a microgravity-induced reduction in monocytes seems a consistent finding, while changes in the number of neutrophils are less consistently reported.

Beyond cell number, functional attributes of leucocytes that are critical to cell migration also appear to be altered by space flight. While the studies in this area are somewhat conflicting, overall they provide strong evidence that microgravity does indeed influence leucocyte function. Most of the data support the concept that neutrophil adhesiveness is increased by microgravity. The levels of adhesion molecules on neutrophils are increased during space flight (64), and a 10-fold increase in chemotactic response of neutrophils exposed to microgravity has been shown. The findings of Boxer *et al*. (65), who showed impaired locomotion of monocytes in modelled microgravity, suggest that the effect is cell specific. Mechanistically, flight stress, and the resulting catecholamine release, may be important to these observed changes in leucocyte function. However, direct exposure of neutrophils to epinephrine had no direct effect on neutrophil adhesion (66); although exposure of endothelial cells to epinephrine decreased neutrophil adherence by 40%, the oxidative functions and microgravity are less well investigated. However, an antiorthostatic suspension (modelled space flight) did not alter the oxidative burst in neutrophils (67).

Another immune cell that might be influenced by space flight is the mast cell. Mast cells release vasoactive amines, which enhance the permeability of regional blood vessels, promoting the passage of solutes and inflammatory cells to wound site. Several studies suggest that mast cells influence inflammation and repair at sites of injury and a link between increased mast cell content and the formation of hypertrophic scars has been suggested (68). To date, the effect of gravity on mast cell function has not been evaluated. However, stress seems likely to affect mast cell function, as steroid-therapy reduces the number of these cells in hypertrophic scars (69). Psychological stress in rats resulted in dura mast cell activation and rat mast cell protease I secretion that were corticotropin-releasing hormone (CRH) dependent (70). Also, it has been proposed that CRH activates skin mast cells leading to vasodilation and increased vascular permeability (71). Although speculative, flight-induced psychological stress might be predicted to cause a sustained hyperactivation of these cells in the wound milieu, with resultant increased hypertrophic scarring. Nonetheless, comprehensive research aiming at the elucidation of the function and number of these cells in microgravity-exposed wounds seems necessary.

Another immune cell type that appears to be functionally impaired by exposure to microgravity is T cells (72–75). The expression of both IL-2 and IL-2Ralpha genes is significantly inhibited in simulated microgravity (73).

Moreover, purified human T lymphocytes are shown to exhibit differential inhibition of transcription factor activation in modelled microgravity. Activation of Activator Protein 1 (AP-1) is blocked with clinorotation, whereas dephosphorylation of nuclear factor of activated T cells occurs (75). While the exact role of T cells in regeneration of damaged tissues is not known, space-induced functional impairments may need to be considered in the context of wounds.

Beyond direct effects on cells themselves, microgravitational exposure may influence the production of the cytokines that connect the cellular elements of wound milieu. Peana *et al*. (76) assessed the effect of microgravity on Prostaglandin E2 (PGE2)-induced oedema and hyperalgesia. Both oedema and hyperalgesia decreased because of anti-inflammatory and anti-hyperalgesic action of simulated microgravity. In contrast, Kumei *et al*. (77) detected enhanced levels of PGE2 in flight samples compared with ground controls in normal rat osteoblast cultures. The secretion of interleukin (IL)-1β, a factor that exerts panoply of effects in wound milieu (78), is almost completely inhibited in microgravity (79). IL-1 is a potent inducer of IL-6. However, despite inhibition of IL-1, the level of IL-6 increased during space flight (80,81). In one report, the expression of IL-2 and IL-2 receptor was significantly suppressed at simulated zero gravity (72,80), although another report failed to detect any alteration of IL-2 level at zero gravity (73). Overall, the available studies of the influence of gravitational stress on cytokine production suggest that microgravity may produce multiple perturbations in secretory patterns at sites of inflammation, such as the healing wound.

Proliferation

In skin, the proliferative stage of healing involves regeneration of epithelial barrier, deposition of extracellular matrix and proliferation of connective tissue cells. Various growth factors secreted during inflammatory stage mediate differentiation and proliferation and function of cellular elements. One key factor is TGF-β, which regulates the deposition of new extracellular matrix through transcriptional activation of genes encoding extracellular matrix molecules such as collagen and proteoglycans (82). TGF-β, can also inhibit tissue protease production and stimulate the secretion of the inhibitors of matrix metalloproteinases (83,84). The majority of the available data indicates that expression of the various isoforms of TGF-β is reduced by exposure to microgravity (85,86), although one study found no such effect (87). The tissue response to TGF-β is decreased under microgravity, suggesting impairment in signal transduction pathways (88). As mentioned above, the influence of space on cytokines such as TGF-β may be simply an indirect effect that stems from flight-induced psychological stress. In support of this concept, glucocorticoids are known to antagonise the effect of TGF-β at the level of transcription (89). Song *et al*. (90) found that glucocorticoids repress TGF-β activation of the TGF-β responsive sequence containing Smad3/4-binding sites. Biomechanical properties of tissue may also influence the modulation of TGF-β function in microgravity, as microgravity generates a low shear strain environment. While shear stress enhances the expression of TGF-β, blocking this mechanical stimulus inhibits its expression (90). Hence, it may be expected that gravity-induced fluid dynamics may lead to downregulation of TGF-β. While extrapolation of these results to regenerating tissues may not be direct, it seems likely that microgravity and the stress of space travel will result in an impairment of both TGF-β production and the cellular response to this cytokine. Given that mice deficient in TGF-β or its signalling components exhibit significant deficits in healing (91), space flight-induced alterations in TGF-β or its signalling pathways would probably have extreme effects on healing. It is known that the biological activity of TGF-β depends mainly on the type of activated receptor/signal transduction pathway and to a lesser extent on the specific isoforms involved. Therefore, we suggest the use of knockout models in microgravity studies of wound healing to efficiently target the alteration in TGF-β signalling cascade and associated modulation of regenerative outcome.

The EGF family, which includes EGF, transforming growth factor-α (TGF-α) and heparin-binding EGF, also seems to be influenced by microgravity (92,93). Several studies suggested that EGFR is important for reepithelialisation, especially during early stages of healing (94,95). EGF and TGF-α appear to be critical to the development of the normal phenotypic features of regenerating epithelium (96,97). However, the deficiency of these growth factors has been reported to be compensated by other growth

Key Points

- beyond direct effects on cells themselves, microgravitational exposure may influence the production of the cytokines that connect the cellular elements of wound milieu
- the available studies of the influence of gravitational stress on cytokine production suggest that microgravity may produce multiple perturbations in secretory patterns at sites of inflammation, such as the healing wound
- the tissue response to TGF-b is decreased under microgravity, suggesting impairment in signal transduction pathways
- we suggest the use of knockoutmodels in microgravity studies of wound healing to efficiently target the alteration in TGF-b signalling cascade and associated modulation of regenerative outcome

Key Points

- the expression of EGF and TGF-a is substantially down regulated and the signal transduction pathways are impaired in microgravity
- space flight-induced psychological stress may lead to down regulation of PDGF and its receptor in wound milieu
- following trauma, rapid immediate closure of gap junctions takes places,uncoupling damaged cells from uninjured ones-directional centripetal migration of keratinocytes into the wound bed is necessary for optimal wound healing
- while sympathetic nervous activity was decreased during head-down bed rest, a similar finding was not observed during microgravity and thus head-down bedrest cannot be applied to simulate changes in sympathoadrenal activity during microgravity
- considering the existing controversy, investigation of microgravity-induced alteration of sympathetic activity and its effect on healing dermal wounds seems necessary
- the contribution of epidermal stem cells to repair of wounded epidermis is now evident
- newly emerging data contradict the proposed negative impacts of microgravity and underline its enhancement of proliferative activity of mesenchymal stem cells

factors such as keratinocyte growth factor (KGF) (98,99). As mentioned previously, the expression of EGF and TGF-α is substantially downregulated and the signal transduction pathways are impaired in microgravity (51–53,100–103). Interestingly, simulated microgravity enhances the activity of KGF (49). Therefore, any impairment of EGF expression that is caused by microgravity might be partially masked through a parallel upregulation of KGF.

PDGF is another factor that must be considered, as PDGF appears to be essential for normal wound healing (104,105). PDGF has two distinct roles in healing procedure: an early function to stimulate fibroblast proliferation and a later function to induce the myofibroblast phenotype (106). Akiyama *et al*. (54) found that the expression of PDGF in microgravity is 62% lower than the control ground samples. Furthermore, the early function of PDGF – the stimulation of fibroblast proliferation – is substantially diminished in this condition (55). Meanwhile, space flight-induced psychological stress may lead to downregulation of PDGF and its receptor in wound milieu (107).

Gap junctional intercellular communications have implicated to play an important role in wound healing (108). Following trauma, rapid immediate closure of gap junctions takes places, uncoupling damaged cells from uninjured ones (109). During reepithelialisation, gap junctions are temporarily lost on the surface of keratinocytes located in the leading edge of centripetal moving rim of epidermis (110). Also, fibroblasts derived from nodules that were excised from Dupuytren's contracture lesions show reduced levels of intracellular gap junctions compared with normal dermal fibroblasts (111). In contrast, promotion of intracellular gap junctions through daily injections of LiCl into polyvinyl alcohol sponge implanted into the wound milieu, enhanced the penetration of granulation tissue into the interstices of the sponge, increased the amount of connective tissue deposited in the surrounding capsule and promoted more organised collagen fibres (112). Liu *et al*. (113) reported that connexin 43 decreased significantly and distributed irregularly after simulated microgravity. On the contrary, Claassen and Spooner could not detect any alteration in channelling activity of cardiac gap junctions following short period microgravitational status (114).

Directional centripetal migration of keratinocytes into the wound bed is necessary for optimal wound healing. Keratinocytes express β2-adrenergic receptor. It has been shown that β-adrenergic receptor activation delays wound healing by preventing the organisation of the actin cytoskeleton and localisation of phosphoextracellular receptor kinase to the lamellipodial edge and its colocalisation with vinculin and thus leading to a considerable delay in reepithelialisation (115,116). Moreover, it has been suggested that β-adrenergic receptor antagonists promote wound reepithelialisation in chronic human skin wound (117). However, β-adrenergic receptor activation enhanced fibroblast proliferation and contraction and meanwhile decreased fibroblast-mediated collagen gel contraction, both of which are detrimental to wound healing (118,119). However, β1- and β2-adrenoceptor blockade impairs cutaneous wound healing, delineating the complicated role of sympathetic system in regulation of healing response (120). Simulation of microgravity through head-down bed rest induced increased responsiveness of sympathetic nervous system through β-adrenergic receptor sensitisation (121). Nonetheless, while sympathetic nervous activity was decreased during head-down bed rest, a similar finding was not observed during microgravity and thus head-down bed rest cannot be applied to simulate changes in sympathoadrenal activity during microgravity (122). Considering the existing controversy, investigation of microgravity-induced alteration of sympathetic activity and its effect on healing dermal wounds seems necessary.

Stem cells from various proximal and distal niches are involved in wound healing. It has been suggested that human mesenchymal stem cells (hMSCs) together with b-fibroblast growth factor accelerate cutaneous wound healing as the hMSCs transdifferentiate into the epithelium (123). The contribution of epidermal stem cells to repair of wounded epidermis is now evident (124,125). Moreover, adult bone-marrow-derived mesenchymal stem cells home the sites of tissue injury and enhance the healing dynamics through differentiating into various cellular elements (126). It has been suggested that microgravity reduces proliferative as well as differentiation capabilities of human mesenchymal stem cells (127,128). However, newly emerging data contradict the proposed negative impacts of microgravity and underline its enhancement of proliferative activity of mesenchymal stem cells (129).

CONCLUSION

It may be concluded that various stages of wound healing and sophisticated interactions between elements involved in healing response are modified in microgravitational status. However, future studies addressing the issue through practical approaches are necessary to understand the aforementioned alterations.

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Key Points

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